Leukemia & Lymphoma, Vol. 44, No. 11 (November 2003), pp. 1843–1853

Taylor&Francis
healthsciences

Multicolor Karyotyping in Acute Myeloid Leukemia

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(Received 20 March 2003)

Cytogenetic data have significantly contributed to our understanding of the heterogeneity of acute myeloid leukemia (AML). In AML, numerous recurrent chromosomal aberrations have been identified, and several of them, e.g. t(8;21)(q22;q22), t(15;17)(q22;q11-12), inv(16)(p13q22), are specific for distinct subgroups. Furthermore, chromosomal aberrations have proved to be of paramount prognostic importance for remission induction and survival. Chromosome analysis using classical cytogenetic banding techniques often fails to completely resolve complex karyotypes and cryptic translocations not identifiable by these techniques have been detected using molecular cytogenetic methods. While fluorescence *in situ* hybridization (FISH) has become an indispensable tool for screening and follow-up of known aberrations, the techniques of spectral karyotyping (SKY) and multiplex-fluorescence *in situ* hybridization (M-FISH) allow for the simultaneous visualization of all chromosomes of a metaphase in a single hybridization step, and thereby enable screening for the aberrations present without their prior knowledge. Therefore, with the introduction of these techniques in 1996 the comprehensive analysis of complex karyotypes and the identification of new, hitherto cryptic translocations and, ultimately, the identification of new disease subgroups seemed possible. Since, more than 600 cases of AML and MDS have been analyzed. Herein, we attempt to summarize the data published and discuss what has been achieved towards realization of these goals.

Keywords: M-FISH; SKY; AML; MDS

INTRODUCTION

Shortly after the introduction of chromosome banding techniques, the t(8;21) was discovered by Rowley in 1972 and was the first aberration to be recognized as a balanced translocation [1]. Since then, numerous other recurrent aberrations have been identified in acute myeloid leukemia (AML) and their detection has become essential for accurate diagnosis and classification of the disease. Furthermore, chromosomal aberrations represent one of the most important independent prognostic factors, influencing the likelihood of remission induction and risk of relapse. Recently, some of these aberrations have been used within the WHO-classification to define specific disease subgroups [2].

Cytogenetic analysis using G- or R-banding (Fig. 1a) still is the most widely used method for identifying chromosome aberrations in leukemic cells. At present, chromosomal aberrations are detected in 50–80% of patients with AML in an age-dependent manner [3–5], thus, a considerable proportion of cases presents with an apparently normal chromosome complement. Furthermore, in approximately one-fourth of cytogenetically aberrant cases of AML the karyotype cannot be fully resolved by banding owing to poorly spread or contracted chromosomes or to the presence of marker chromosomes, rings, or unidentified material attached to a chromosome. Consequently, there has been considerable effort to develop techniques that would facilitate metaphase screening and allow for the karyotypic analysis of nondividing cells. Since the late 1980s, fluorescence *in situ* hybridization (FISH) techniques have been developed and used as adjunct to classical cytogenetic methods (Fig. 1c). FISH has allowed for the analysis of interphase nuclei, as well as for the confirmation of aberrations suspected by banding analysis. Furthermore, FISH has become an important tool for follow-up analysis. While FISH requires prior knowledge of the aberrations,

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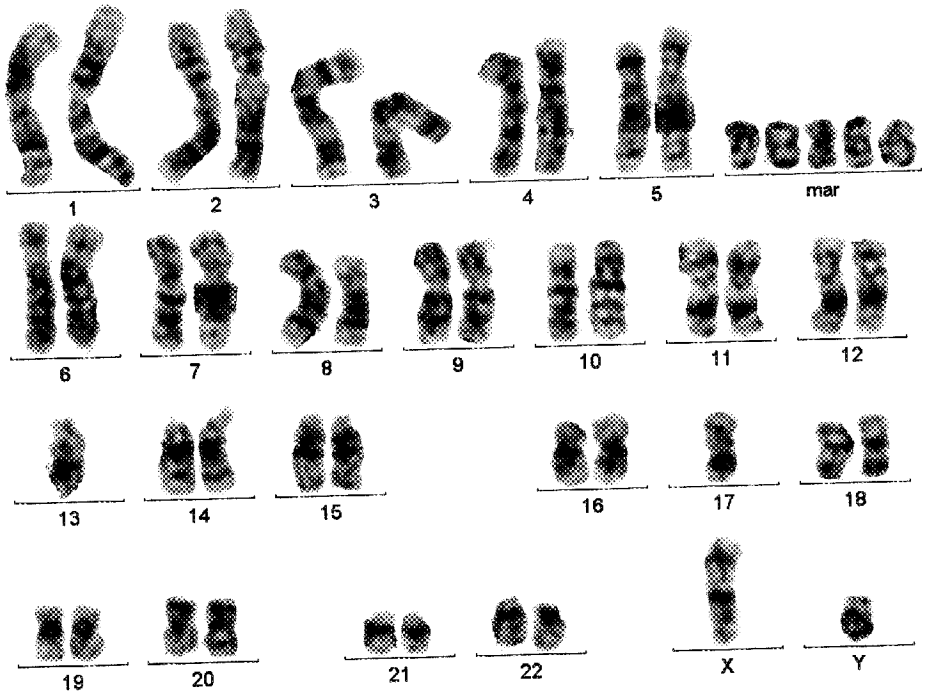
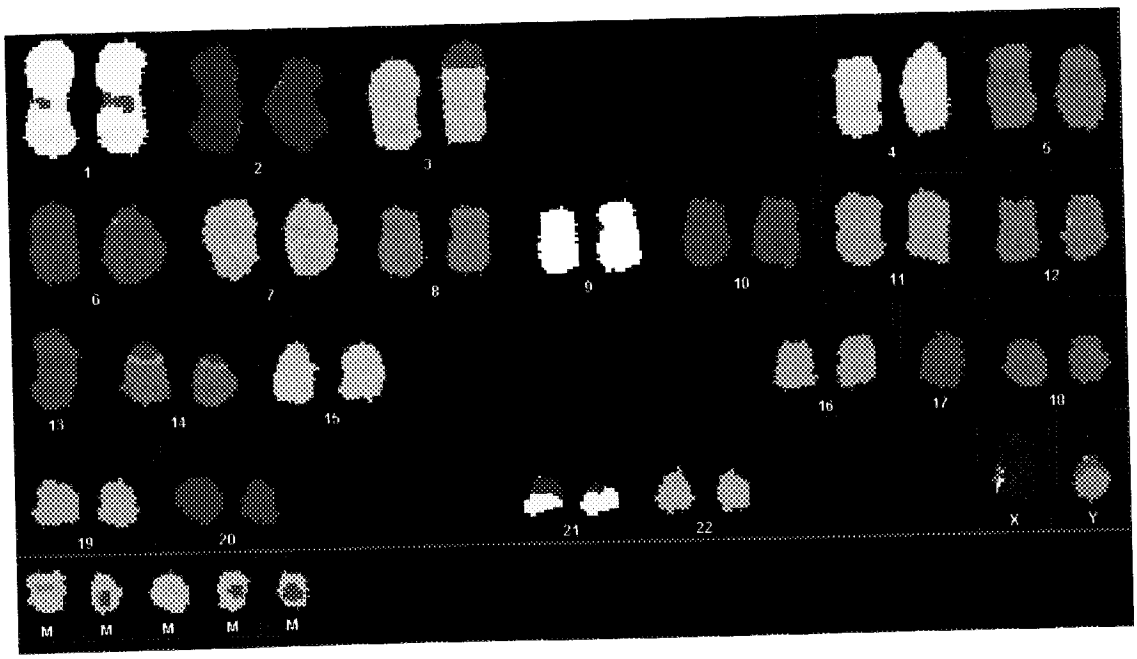
ISSN 1042-8194 print/ISSN 1029-2403 online © 2003 Taylor & Francis Ltd
DOI: 10.1080/1042819031000160360 5

a)



b)



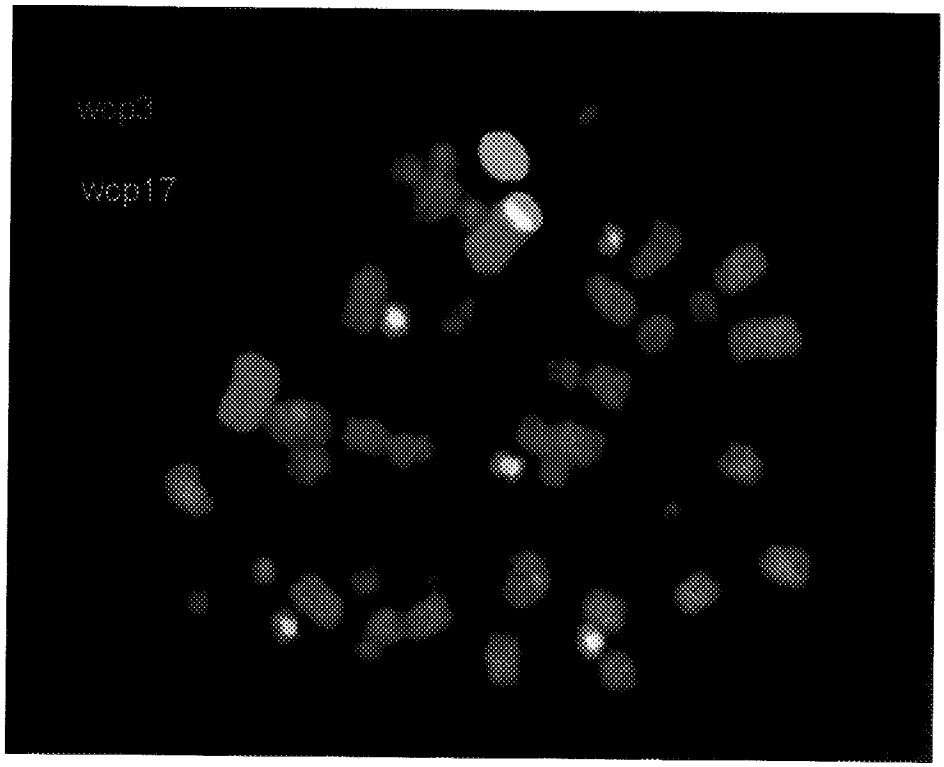


FIGURE 1 a: Complex karyotype of a patient with AML-M5 after G-banding. Five marker chromosomes were not identified (mar). b: Karyotype of the same patient after SKY classification. The marker chromosomes were characterized as derivative chromosomes 17. The centromere consisted of material derived from chromosome 17, the p and q-arm showed the classification color of chromosome 3. c: SKY results were confirmed using painting probes for chromosome 3 and 17.

multicolor karyotyping techniques enable screening for the aberrations present without such knowledge. The development of spectral karyotyping (SKY) and multiplex-FISH (M-FISH) has therefore allowed for a comprehensive analysis of cases with complex aberrations and encouraged the hope for the detection of chromosomal aberrations in cases with a seemingly normal karyotype. With such technical developments, the identification of new, recurrent chromosomal rearrangements that might define new subgroups, such as the t(12;21)(p13;q22) in childhood B-ALL, seemed potentially attainable.

Here, we review the data published and discuss what has been achieved towards the realization of the aforementioned goals. Unfortunately, it is beyond the scope of this review to include all cases published. We have, therefore, focused on publications reporting possible recurrent aberrations or those comprising several cases and have not included cell lines. Most series did not only include cases with *de novo* AML, but also MDS and AML developing from MDS, as well as in some instances therapy-related cases. For the purpose of this review, it was not useful to separate these cases.

METHODS

SKY and M-FISH are FISH based methods that allow for the simultaneous display of all chromosomes in different colors using five fluorochromes, alone and in combination in a single experiment [6,7]. The techniques employ a different approach to image acquisition: SKY requires a single exposure and uses a combination of an interferometer, CCD imaging and successive Fourier transformation; M-FISH employs five fluorochrome-specific filters, sequential image acquisition with a CCD camera, and subsequent overlay of these images. Figure 1 depicts the SKY classification of a case of AML-M5. The strength of these techniques is the immediate identification of interchromosomal aberrations, for example, translocations that lead to a color difference on the derivative chromosome, as well as the elucidation of complex rearrangements. They have their limitations in the identification of chromosomal changes that do not lead to a discernable color change: small deletions, duplications and intrachromosomal inversions, which can only be identified in conjunction with the inverted DAPI-image or the G-banded karyotype. The resolution of SKY for the detection of interchromosomal rearrangements has been shown to be between 500–2000 Kb, but of course significantly depends on metaphase chromosome extension as well as hybridization quality [6,8]. Similarly, translocated material of 1–2,6 Mb has proved difficult to detect using M-FISH [9,10]. To achieve the most comprehensive karyotype description possible a combination of the techniques available is warranted.

For further new FISH-based techniques see, e.g. Chudoba *et al.*, [11], Kearney [12,13], Schröck and Padilla-Nash [14], Fauth *et al.*, [15], Liehr *et al.*, [16].

PUBLISHED DATA

To date, over 600 cases of AML and MDS have been analyzed by SKY or M-FISH. Table I gives a summary of these reports. The first study on hematological malignancies was undertaken by Veldman *et al.*, [17]. This study demonstrated that archived cell pellets originally prepared for routine cytogenetic analysis could be used for SKY and included 7 AML and MDS cases with chromosome aberrations not completely identifiable by conventional banding analysis. In these cases, SKY recognized previously unidentified material, detected subtle translocations and clarified complex aberrations and thereby demonstrated its value for the analysis of such cases [17]. After the feasibility and usefulness of multicolor karyotyping was amply demonstrated in several studies on AML and MDS [18–24], different approaches to case selection were taken in the larger studies published. While in most instances, cases were selected according to their cytogenetic characteristics, i.e. cases with either normal or complex karyotypes, or with aberrations involving a specific chromosomal region, their morphological characteristics, the patients age or therapy-relatedness have also been used as selection criteria.

Findings in Cases with Normal G-banded Karyotype

The great majority of cases presenting with a normal karyotype remained normal after SKY-analysis [23–26] as well as M-FISH analysis [27–33]. Nevertheless, in the series of 28 patients with normal karyotype reported by Zhang *et al.*, [25], aberrations not identified by banding analysis were detected in two cases (7%). Both aberrations detected, a cryptic t(11;19) and a monosomy 7 in a minor clone, are associated with a poor prognosis. Interestingly, a cryptic t(11;19) was detected by SKY/M-FISH in three further cases [20,26,28], indicating that this translocation can be missed by conventional banding analysis. The detection of small clones with aberrations of known unfavourable prognostic impact, -7 and del(5q), has been reported in two cases [25,26]. However, the relevance of such findings remains to be determined. To date, no new recurrent translocation has been detected by SKY/M-FISH in the group of patients presenting with a normal karyotype.

Findings in Cases with a Complex Karyotype

Recently, several studies have focused exclusively on the analysis of cases presenting with complex karyotypes [34–39]. In these cases, the overwhelming majority of aberrations detected or redefined using SKY/M-FISH analysis were unbalanced translocations as opposed to balanced ones. The consequence of unbalanced aberrations frequently is a loss of chromosomal material, and common losses of 5q, 7q, and 17p were confirmed in all series. Overall, the region by far most frequently lost was 5q, e.g. reported in [illegible] of cases, respectively, by Schoch *et al.*, and Van Limbergen *et al.*, [38,39]. In addition, the unbalanced aberrations detected also resulted in a recurrent partial loss of 12 p [23,32,34, 35,37–39]. Less frequently lost were segments 11p, 13q, 16q, 17q, and 20q [23,26,37–39].

Whereas a monosomy 5 described by G-banding in most instances was recognized to be a deletion after SKY analysis, confirmed true monosomies most frequently implicated chromosome 7 in all series, as well as chromosomes 18, 17, and 16, respectively [34,35,37–39].

Overall, loss of chromosomal material seemed more common than gains/amplifications, but a gain/amplification of 11q involving the *MLL* gene was a consistent finding [32,36–39]. Van Limbergen *et al.*, grouped their cases according to the most frequent aberrations found in their series: $-5/5q-$, $-7q$, 3q rearrangements, and *MLL* gain or amplification (irrespective of the additional presence or absence of chromosome 5 rearrangements in the latter). There was a trend towards poorer survival in patients with one versus two or more of these aberrations. Furthermore, the presence of *MLL* copy number gain or amplification concurrently with 5q-aberrations was significantly related with an extremely short survival time [39].

Another segment frequently found to be overrepresented was 21q [26,36–38]. It was the segment most frequently gained in the cases analyzed by Mrózek *et al.*, (8/29 patients). Interestingly, this was seen in 7/8 patients diagnosed with *de novo* AML. In these cases, the copy number gain of the *RUNX1* (*AML1*) gene did not correlate with the amount of 21q material gained, excluding this gene as the target of amplification in these cases. Such a correlation was only found in one patient presenting with secondary AML (from MDS), but two similar cases with secondary AML have been described in previous studies [20,26]. Mrózek *et al.*, therefore, speculated about a possible role for such aberrations in disease progression from MDS to AML.

Further chromosomal regions frequently gained/amplified were 8q, 22q [37,38]. In a recent comparison of M-FISH and CGH analysis in 41 patients with a complex aberrant karyotype chromosomal regions most often gained and lost were narrowed down (lost: 5q31.1q31.3, 17p13, 7q32q35, 18q21q22, 12p13, 16q22q24; gained:11q23q25, 1p33p36, 8q22q24) [32].

In one and two cases, respectively, amplification of 11q not involving *MLL* and 19q11-13 was seen in cases with DMIN [40].

Findings in Patients with Aberrations Involving a Specific Chromosomal Region

Although these studies were primarily designed as FISH studies, SKY provided additional information in the cases analyzed. In a study by Ning *et al.*, on cases with terminal 5q-deletions (as determined by banding analysis), the use of subtelomeric probes revealed in six of seven cases that these deletions were not terminal but interstitial.

TABLE I SKY or M-FISH studies in AML

Reference	Disease*	No. of Cases (AML + MDS)	Karyotype after G-/Q-banding	Results after SKY or M-FISH analysis
Veldman *et al.* [17]	AML + MDS	3 + 4	ABNC, Complex	SKY identified material, marker and ring chromosomes not recognizable by G-banding in all cases, clarification of complex rearrangements
Beverloo [18]	AML + MDS	40 + 6	Normal, ABNC, Complex	SKY: 25/25 cases with NK: no hidden aberrations detectable 14 /21 cases: karyotype description extended. Next to involvement of chromosome 5, regions of chromosomes 2, 3, 4, 12 and 22 were participating in the new abnormalities.
Kakazu *et al.* [20]	AML + MDS	4 + 16	Normal, ABNC, Complex	SKY: 1/2 cases normal, 1 case cryptic t(11;19); 18 cases: identification of material previously unidentified, detection of five translocations involving apparently normal chromosomes
Calabrese *et al.* [21]	AML	5	ABNC, Complex	SKY: Clarification of complex rearrangements and detection of cryptic abnormalities
Helias *et al.* [22]	AML + MDS	10 + 20	ABNC, Complex	SKY: Precise identification of chromosomes involved in translocations
Mohr *et al.* [23]	AML + MDS	32 + 7	Normal, ABNC, Complex	SKY: NK: no concealed aberrations detectable in 19/19 cases, 13/20 cases with aberrations: more comprehensive karyotype description
Zhang *et al.* [25]	AML	28 7 (+7 other)	Normal Validation cases	SKY: 26/28 normal, cryptic t(11;19) and −7 (3 of 21 MP) in 1 case each; t(6;11), t(6;14) not always recognized as balanced, classification of DMIN ambiguous
Andersen *et al.* [27]	t-AML + t-MDS	54	Normal, cases with unidentified material	M-FISH: 11/11 with NK remained normal, 43 cases with unidentified material: frequent loss of 5q, 7q, gain of 11q 19/43 cases: dicentric chromosomes resulting in loss of 5q, 7q, 17p
Barouk-Simonet *et al.* [36]	AML + MDS	8 + 12	Complex	14/20 loss 17p and *P53* deletion, 3/20 11q23 and *MLL* amplification, 2/20 21q22 and *AML1* amplification, 3 translocations involving 19q13
Hilgenfeld *et al.* [26]	AML-M2	37	Normal, ABNC, Complex	SKY: 4/18 cases with NK: cryptic t(11;19), del(5q), der(21)t(18;21) and DMIN, −19+−21, in one case, respectively; DMIN classification ambiguous 9/19 cases: modification of karyotype description, 5 cases: partial gain of chr 21, 3 cases: *MYC* amplification
Kerndrup *et al.* [24]	AML	35	Normal, ABNC, Complex	SKY: 8/8 cases with NK: no hidden aberrations detectable 11/12 cases with single clonal aberration confirmed, 9/10 cases with CK: karyotype description extended
Lindvall *et al.* [34]	AML + MDS	12 + 10	Complex	Classification of marker chromosomes, redefinement of multiple chromosome rearrangements
Odero *et al.* [35]	AML + MDS + biphenotypic AL	11 + 6 + 1	Complex	G- Vs SKY: concordant in 3 cases Rest: identification of hidden translocations and reconstruction of complex rearrangements
Brown *et al.* [28]	AML	27	Normal or with isolated trisomy	23/27 age ≤ 14 y M-FISH: 27/69 cases analysed, one case with t(11;19), Telomeric probes (M-TEL): 2 cases with t(5;11), not detected by M-FISH
Dalley *et al.* [29]	AML (age ≧ 60 y)	18	Normal, ABNC, Complex	M-FISH: confirmed karyotype in 15 patients (including all 8 patients with NK), clarified karyotype in 2 patients, and failed to detect a t(12;17)(p12;p13) in 1 patient.

TABLE I – *continued*

Reference	Disease*	No. of Cases (AML + MDS)	Karyotype after G-/Q-banding	Results after SKY or M-FISH analysis
Klaus *et al.* [31]	AML	25	Normal	M-FISH: 25/489 patients analysed, in 1/25 cases t(17;21)(p11;q11) FISH-analysis of all cases: approx. 3 % with clonal aberrations
Mrozek *et al.* [37]	AML	29	Complex	Hidden overrepresentation of 21q, 11q, and 22q, 9 novel balanced translocations identified by SKY
Sait *et al.* [40]	AML + MDS	8 + 1	DMIN	Amplification of material from chr 11 (not *MLL*) in one case, from chr 19 in 2 cases, FISH: 19q11-q13.1; 4/9 cases *MYC* amplification
Schoch *et al.* [38]	AML	125	Complex	M-FISH: most frequently lost: 5q, 17p, 12p; gained: 11q, 21q, 8q
Schoch *et al.* [32]	AML	41 (250)	Complex	M-FISH/CGH comparison: regions lost: 5q31.1-q31.3, 17p13, 7q32-q35, 18q21q22, 12p13, 16q22q24; gained:11q23q25, 1p33p36, 8q22q24
Van Limbergen *et al.* [39]	AML + MDS	23 + 13	Complex	Cytogenetic subgroups: −5/5q−, (+/−5q): del(7q), 3q-rearrangements, MLL gain/amplification
Vey *et al.* [33]	AML	12	Normal	M-FISH: all cases remained normal

Abbreviations: AL, acute leukemia; AML, acute myeloid leukemia; ABNC, aberrant but not complex; CK, complex karyotype; G-, G-banding; MDS, myelodysplastic syndrome; MP, metaphase; NK, normal karyotype; Q-, Q-bandin.
The table lists only studies including more than 3 cases analyzed by SKY/M-FISH. Recurrent aberrations detected in these series have been summarized in Table II.
* Most series included cases with *de novo* as well as secondary AML, in some series therapy-related cases also included.

In the remaining case, SKY identified a cryptic t(5;12), confirming that none of the supposed terminal deletions were indeed terminal [41]. In the series of Odero *et al.*, nine (four AML, three MDS, two other) of 15 patients with 12p rearrangements had an *ETV6* rearrangement recognized using FISH. SKY verified FISH results and further characterized aberrations in two cases with complex karyotypes. Six new *ETV6* partner bands (1p36, 4q22, 6p21, 6q25, 12q24, 17q12) were identified in this study [42].

Findings in Studies using Other Selection Criteria

Hilgenfeld *et al.*, had selected cases diagnosed morphologically as having AML-M2 according to the FAB-classification, given that this is one of the most frequent subgroups of AML and only a subset of patients present with a t(8;21). Yet, no new balanced recurrent chromosomal aberration associated with this morphologic phenotype was detected by SKY analysis. A partial gain of chromosome 21 in 5/37 cases was detected in this series and in 4/5 cases these aberrations resulted in a *RUNX1* copy number gain. Three of these five cases presented with a complex karyotype. Furthermore, an amplification of *MYC* was detected in three cases [26].

In a series investigating cases with therapy-related MDS/AML, 43 cases with unidentified aberrations by G-banding showed similar findings as reported for complex cases. Dicentric chromosomes were detected in 19/43 cases resulting in a loss of 5q, 7q, 17p [27].

The study of Dalley *et al.*, [29] was designed for AML patients over 60 years. Conventional G-banded analysis was performed in all 28 patients prior to evaluation with CGH and M-FISH. CGH was performed in 15 patients. Metaphase preparations from 18 patients (10 with abnormal karyotypes) were analysed by M-FISH, five of these patients were also analysed by CGH. M-FISH confirmed karyotype in 15 patients, and provided additional information on two patients with a complex karyotype, but failed to detect a telomeric translocation in one patient.

Possible Recurrent Aberrations Reported

Balanced Aberrations

While only the minority of aberrations detected by multicolor karyotyping were balanced, reciprocal translocations newly detected or redefined by SKY or M-FISH have been described in nearly every study published. For most of the novel translocations detected it is unclear at present if they constitute recurrent events in AML; possible recurrent translocations reported are summarized in Table II.

A t(2;4)(p23;q31) revised by SKY in the series of Mrózek *et al.*, [37] has been reported once previously in a case of AML-M2 as a sole aberration [43]. Furthermore, Van Limbergen *et al.*, [39] identified two balanced translocations in their series which they suggested as

possible recurrent aberrations: a t(4;5)(q31;q31) and [−4,der(5)t(4;5)(q31;q31)] were identified in two cases of AML-M6, respectively, and a t(1;8)(p31;q22) was redefined in a patient with MDS-RAEBt. Involvement of *ETO* in the latter case was excluded. Three translocations involving chromosomes 1 and 8 and similar breakpoints have previously been reported. Fine-mapping of the 5q31 breakpoints in the first two cases using region-specific PAC- and BAC-clones revealed that they differed by approximately 3 Mb [39]. Two translocations, t(2;3)(p23;q27) and t(12;22)(p13;q12-13) were confirmed by SKY in the series of Mrózek *et al.*, [37] and have recently been recognized as recurrent events in AML [4].

To our knowledge, two novel aberrations involving new translocation partners of *RUNX1*, a t(3;21)(p13 or p?25;q22) and a t(7;21)(p22;q22), as well as one translocation involving *EVI1*, t(3;6)(q26;q25), have been reported [37,39,44]. Additionally, a t(3;15)(q26;q24) was detected by SKY in a case of AML-M1 developed from MDS in the series of Kakazu *et al.*, [20] and has not been reported previously. Nevertheless, it was not investigated if *EVI1* was involved in this translocation.

Unbalanced Aberrations

The recurrent unbalanced aberrations reported are summarized in Table II.

In the series of hematological cases analyzed by Veldman *et al.*, [17], a der(7)t(7;14)(q22;q1) was described in one case with AML and MPD and a complex karyotype. This aberration was since detected in two other cases presenting with complex karyotypes [23,39]. Interestingly, in all three cases reported, one copy of chromosome 14 was missing allowing for the possibility that the der(14) was lost and that this indeed represents a reciprocal aberration. A reciprocal t(7;14)(q3?1;q2?2) was described in another case in the series of Mohr *et al.*, [23]; however, the assigned breakpoints differ. In addition, the cases described by Veldman *et al.*, [17] as well as both cases in the series of Mohr *et al.*, [23] also carried a trisomy 8.

In addition, balanced translocations with the same breakpoints as detected in two other unbalanced aberrations, der(12)t(12;17)(p13;q21) and der(5)t(5;17)(q11;q11), have been reported. While a t(12;17)(p13;q21) is a recurrent aberration in ALL, one case of AML with a balanced t(5;17)(q11;q11) has been reported. Furthermore, in several of the cases with a der(5)t(5;17)(q11;q11) one copy of chromosome 17 was lost [45].

TABLE II Possible recurrent balanced and unbalanced translocations as well as translocations involving known genes detected by multicolour karyotyping

Recurrent balanced aberrations	References
t(1;8)(p31;q22)	[39]
t(2;3)(p23;q27)	[4,37]
t(2;4)(p23;q31)	[37,43]
t(4;5)(q31;q31)	[39]
t(12;22)(p13;q12-13)	[4,37]
Recurrent unbalanced aberrations	
der(1)t(1;19)(p13;p13.1)	[66]
der(5)t(5;17)(q11;q11)	[19,37]
der(5;17)(p10;q10)	[37,39]
dic(5;17)(q11;p11.2)	[38,67]
der(7)t(7;14)(q22;q1),+8, −14	[17]
der(7)t(7;14)(q22;q1?2),+8, −14	[23]
der(7)t(7;14)(q21;q13), −14	[39]
der(12)t(12;17)(p13;q21)	[26,37]
der(16)t(11;16)(q13;q24)	[37,68]
der(17)t(5;17)(p11;p11.2)	[26,67]
dic(17;20)(p11;q11)	[38]
der(18)t(18;21)(p11.2;q11.2)	[20,26]

Multicolor Karyotyping of Mouse Models of AML

Karyotyping mouse chromosomes has been challenging in the past, as all mouse chromosomes are acrocentric and of similar size. SKY has been developed for mouse chromosomes and has already proved to be a very useful tool in the analysis of mouse models of human cancer ([46], for review see [47]). Recently, karyotyping mouse chromosomes by multiplex-FISH has also become available [48].

Castilla *et al.*, reported that a high percentage of $Cbfb^{+/Cbfb-MYH11}$ chimaeras (*Cbfb*-MYH11, inv(16)-fusion protein) developed acute myelomonocytic leukemia after ENU (N-ethyl-N-nitrosourea)-mutagenesis. SKY-analysis of leukemic cells from four of these cases did not reveal any chromosomal changes [49]. In contrast, SKY-analysis of leukemic cells derived from transgenic mice expressing *PML-RARα* or *PML-RARα/RARα*-PML, *PML-RARα* or *PML-RARα/BCL2* as well as *PLZF-RARα* or *PLZF-RARα/RARα-PLZF* revealed distinct recurrent chromosomal aberrations [50–52]. In part, the aberrations found resemble those of the human disease, e.g. an additional mouse chromosome 15 was a recurrent finding. Mouse chromosome 15 contains regions orthologous to part of human chromosome 8q encompassing the location of *MYC* [52].

WHAT HAS BEEN ACHIEVED?

Comprehensive Analysis of Komplex Karyotypes

The numerous reports published, not only on the analysis of hematological malignancies but also of solid tumors as well as of constitutional abnormalities, have amply demonstrated the power of multicolor karyotyping to elucidate complex karyotypes. Still, additional FISH experiments are often necessary to clarify and confirm ambiguous results. And limitations have been reported regarding the unambiguous identification of small marker chromosomes as well as DMIN, possibly due to their low euchromatin content [17,23,25,26].

In AML, while the vast majority of cases presenting with a normal karyotype remained normal, the comprehensive analysis of complex karyotypes has led to

the identification of several recurrent balanced as well as unbalanced aberrations. However, it is most likely that not all recurrent aberrations have been recognized as yet, as not all karyotypes are published in detail, and data analysis, especially of the large number of unbalanced translocations detected, is difficult.

Multicolor karyotyping has also enabled a more accurate assessment of chromosomal gains and losses than possible by G-banding, thereby confirming recurrent losses and identifying recurrent gains/amplifications. Some of the recurrent gains and amplifications recognized potentially identify new subgroups (see below).

Identification of Cryptic Translocations

Although to date no new recurrent reciprocal translocation with a frequency similar to the t(12;21) in B-ALL has been identified in AML, many novel, some new and possibly recurrent balanced aberrations as well as some new rearrangements involving previously recognized breakpoints/genes have been detected. However, the majority of aberrations redefined or newly identified by multicolour karyotyping were unbalanced. While most of these unbalanced rearrangements identified undoubtedly are unbalanced, in some instances one of the derivative chromosomes may have been missing or the balanced nature of the aberration might not have been detected.

Using SKY, evidence has accumulated in several studies that the detection of subtelomeric regions (reported for 6q27 → qter, 9q34 → qter, 11p15 → pter, 12p13 → pter, 14q32 → qter, 18p11.32 → pter, 18q23 → qter) is limited and that reciprocal translocations therefore appeared to be unbalanced, were not detected or were not unambiguously identifiable or misclassified [8,25,53–55]. This also holds true for M-FISH [56].

The reasons for this being a very low fluorescence intensity of, e.g. 9q34, and overlapping fluorescence, fluorescent "flare", at the interface between juxtaposed translocated chromosomal material [14,55,56].

Given the technical limitations of both methods, it seems very possible that some reciprocal rearrangements have not been recognized as such. To remedy these limitations, it has been suggested to supplement the SKY probe cocktail with subtelomeric probes or microdissection probes of specific bands [14,25]. Tonon *et al.*, [57] supplemented the SKY kit with gene specific FISH probes and showed that this in principle is a feasible approach. For M-FISH, new labelling strategies to improve the M-FISH probe set as well as the combination with multiplex-labeled region or locus-specific probes have been developed [56,58]. Besides, FISH-based assays for the simultaneous detection of subtelomeric regions have been developed [59–61].

A recent study by Brown *et al.*, proved that the use of subtelomeric probes is fruitful as a cryptic t(5;11)(q35;p15) in two out of 69 cases of childhood AML (61 cases with normal karyotypes, 8 cases with isolated trisomy) using a set of subtelomeric probes. In both cases, the translocation was not seen with M-FISH [28].

Identification of New Disease Subgroups

Another important goal has been and continues to be the identification of subgroups of prognostic relevance in order to develop better "tailored" therapeutic approaches.

A gain or amplification of 11q and *MLL* as well as, unexpectedly, of 21q were recurrent findings predominantly in patients with complex karyotypes [26,36–39]. While amplification of *MLL* occurring concurrently with a deletion of 5q was associated with an extremely poor prognosis in the retrospective analysis of Van Limbergen *et al.*, so far no difference in survival between *de novo* AML cases with and without 21q gain was seen [37,39]. Nevertheless, the number of cases was small and further investigation of such cases is warranted.

MYC amplification has been reported to be associated with poor survival in cases with complex karyotypes [62]. While a gain of 8q was found in more than 30% in the series reported by Schoch *et al.*, [38], an amplification of *MYC* was less frequently detected [26,37,40]. Interestingly, in a large study on *de novo* AML isolated trisomies of chromosomes 8, 11, 13 and 21 have recently been acknowledged as adverse prognostic factors [63].

It is well known and has been confirmed by multicolour karyotyping analysis that loss of 5q, 7q, as well as 17p frequently occurs in cases with complex karyotypes. In their series, Schoch *et al.*, [38] analyzed the frequency of cases presenting with deletions of all three, two, only one or none of those regions (concurrent loss of 5q, 7q and 17p in 24% of the cases, 26% deletion of chromosomes 5 and 17p, 18% deletion of chromosomes 5 and 7, 15% deletion of chromosome 5, 10% no involvement of these regions). Unfortunately, it was not investigated, whether these patterns correspond to subgroups with a difference in survival within this group presenting with a complex karyotype and an already poor outcome.

With the analysis of more cases using multicolor karyotyping, especially those presenting with monosomies detected by banding that are likely not to be true chromosome losses, e.g. −5, −20, −21, and subsequent survival analysis, more distinct patterns as well as relevant subgroups will hopefully emerge.

CONCLUSIONS

Since its introduction, multicolour karyotyping has proved to be a very useful molecular cytogenetic tool, especially for the clarification of complex karyotypes. The combination of classic cytogenetic banding techniques with molecular cytogenetic tools allows for a characterization of chromosomal aberrations with unprecedented accuracy. Thereby, multicolour karyotyping has enabled a more accurate assessment of gains and losses than possible by G-banding and possible new subgroups

have started to emerge. While many novel, some new and possibly recurrent balanced aberrations have been detected, difficulties in the detection of translocations involving small subtelomeric segments do exist. These shortcomings can be overcome by improved probe sets and/or the additional use of further techniques, e.g. assays employing subtelomeric probes. This will eventually lead to the detection of further balanced rearrangements.

To date, over 160 structural rearrangements have already been recognized as recurrent in AML (reviewed by Mrózek *et al.* [4]), however, this "list" is not complete. With the wealth of data generated by SKY and M-FISH, it is currently virtually impossible to assess all the data for recurrent aberrations, especially unbalanced ones. Furthermore, not all karyotypes are published in detail. Hopefully, with a broader use of the available databases as well as the recently developed SKY and CGH database [64], accessibility and data mining will be significantly facilitated and lead to the identification of additional recurrent aberrations as well as aid the characterization of further new subgroups.

The use of additional tools that have been developed, like the set of BAC-clones available through the Cancer Chromosome Aberration Project (CCAP), enables fine-mapping of (recurrent) breakpoints and thereby rapid identification of target genes located in the vicinity [65]. The use of such clones for array-CGH to further characterize amplified as well as deleted chromosomal regions may also prove to be successful in AML.

Furthermore, the analysis of murine models for AML will afford clues for the delineation of secondary aberrations relevant for leukemogenesis. Identifying further recurrent chromosomal aberrations, characterizing and investigating the chromosomal regions gained and lost, and thereby defining possible new disease subgroups will continue to provide important groundwork for a better understanding of the biology of AML, and hopefully lead to the development of more specific and effective therapeutic strategies.

Acknowledgements

The authors thank Turid Knutsen and Hesed Padilla-Nash for critically reading the manuscript.

References

[1] Rowley, J.D. (1973) "Identification of a translocation with quinacrine fluorescence in a patient with acute leukaemia", *Annals of Genetics* **16**, 109–112.

[2] Harris, N.L., Jaffe, E.S., Diebold, J., Flandrin, G., Muller-Hermelink, H.K., Vardiman, J., Lister, T.A. and Bloomfield, C.D. (1999) "World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues; Report of the clinical advisory committee meeting—Airlie House, Virginia, November 1997", *Journal of Clinical Oncology* **17**, 3835–3849.

[3] Mrózek, K., Heinonen, K., de la Chapelle, A. and Bloomfield, C.D. (1997) "Clinical significance of cytogenetics in acute myeloid leukemia", *Seminars in Oncology* **24**, 17–31.

[4] Mrózek, K., Heinonen, K. and Bloomfield, C. (2001) "Clinical importance of cytogenetics in acute myeloid leukemia", *Best Practice & Research Clinical Haematology* **14**, 19–47.

[5] Grimwade, D., Walker, H., Oliver, F., Wheatley, K., Harrison, C., Harrison, G., Rees, J., Hann, I., Stevens, R., Burnett, A. and Goldstone, A. (1998) "The importance of diagnostic cytogenetics on outcome in aml: analysis of 1,612 patients entered into the MRC AML 10 Trial. The medical research council adult and children's leukaemia working parties", *Blood* **92**, 2322–2333.

[6] Schröck, E., du Manoir, S., Veldman, T., Schoell, B., Wienberg, J., Ferguson-Smith, M.A., Ning, Y., Ledbetter, D.H., Bar-Am, I., Soenksen, D., Garini, Y. and Ried, T. (1996) "Multicolor spectral karyotyping of human chromosomes", *Science* **273**, 494–497.

[7] Speicher, M.R., Ballard, S.G. and Ward, D.C. (1996) "Karyotyping human chromosomes by combinatorial multi-fluor FISH", *Nature Genetics* **12**, 368–375.

[8] Fan, Y.S., Siu, V.M., Jung, J.H. and Xu, J. (2000) "Sensitivity of multiple color spectral karyotyping in detecting small interchromosomal rearrangements", *Genetic Testing* **4**, 9–14.

[9] Holinski-Feder, E., Reyniers, E., Uhrig, S., Golla, A., Wauters, J., Kroisel, P., Bossuyt, P., Rost, I., Jedele, K., Zierler, H., Schwab, S., Wildenauer, D., Speicher, M.R., Willems, P.J., Meitinger, T. and Kooy, R.F. (2000) "Familial mental retardation syndrome ATR-16 due to an inherited cryptic subtelomeric translocation, t(3;16)(q29;p13.3)", *American Journal of Human Genetics* **66**, 16–25.

[10] Jalal, S.M. and Law, M.E. (2002) "Multicolor FISH", *Methods in Molecular Biology* **204**, 105–120.

[11] Chudoba, I., Plesch, A., Lorch, T., Lemke, J., Claussen, U. and Senger, G. (1999) "High resolution multicolor-banding: a new technique for refined FISH analysis of human chromosomes", *Cytogenetics and Cell Genetics* **84**, 156–160.

[12] Kearney, L. (1999) "The impact of the new fish technologies on the cytogenetics of haematological malignancies", *British Journal of Haematology* **104**, 648–658.

[13] Kearney, L. (2001) "Molecular cytogenetics", *Best Practice & Research Clinical Haematology* **14**, 645–668.

[14] Schröck, E. and Padilla-Nash, H. (2000) "Spectral karyotyping and multicolour fluorescence *in situ* hybridisation reveal new tumor-specific chromosomal aberrations", *Seminars in Hematology* **37**, 334–347.

[15] Fauth, C. and Speicher, M.R. (2001) "Classifying by colors: FISH-based genome analysis", *Cytogenetics and Cell Genetics* **93**, 10, Cytogenetics and Cell Genetics, 94, 254.

[16] Liehr, T., Weise, A., Heller, A., Starke, H., Mrasek, K., Kuechler, A., Weier, H.U. and Claussen, U. (2002) "Multicolor chromosome banding (MCB) with YAC/BAC-based probes and region-specific microdissection DNA libraries", *Cytogenetic and Genome Research* **97**, 43–50.

[17] Veldmann, T., Vignon, C., Schröck, E., Rowley, J.D. and Ried, T. (1997) "Hidden chromosome abnormalities in haematological malignancies detected by multicolour spectral karyotyping", *Nature Genetics* **15**, 406–410.

[18] Beverloo, H.B., van Drunen, E., Smit, B. and Slater, R. (1999) "Identification of new chromosomal aberrations in acute leukemia using spectral karyotyping", *Blood* **94**, 494a.

[19] Fleischman, E.W., Reshmi, S., Sokova, O.I., Kirichenko, O.P., Konstantinova, L.N., Kulagina, O.E., Frenkel, M.A. and Rowley, J.D. (1999) "Increased karyotype precision using fluorescence *in situ* hybridisation and spectral karyotyping in patients with myeloid malignancies", *Cancer Genetics and Cytogenetics* **108**, 166–170.

[20] Kakazu, N., Taniwaki, M., Horiike, S., Nishida, K., Tatekawa, T., Nagai, M., Takahashi, T., Akaogi, T., Inazawa, J., Ohk, M. and Abe, T. (1999) "Combined spectral karyotyping and DAPI banding analysis of chromosome abnormalities in myelodysplastic syndrome", *Genes, Chromosomes, and Cancer* **26**, 336–345.

[21] Calabrese, G., Fantasia, D., Spadano, A., Morizio, E., Di Bartolomeo, P. and Palka, G. (2000) "Karyotype refinement in five patients with acute myeloid analysed using spectral karyotyping", *Haematologica* **85**, 1219–1221.

[22] Helias, C., Ferrant, D., Leymarie, V., Aurich-Costa, J., Cherif, D. and Lessard, M. (2000) "Assessment of IPM-FISH, a new M-FISH technique combining combinatorial labeling and R-banding: Study of a serie of 50 various hemopathies", *Blood* **96**, 707a.

[23] Mohr, B., Bornhäuser, M., Thiede, C., Schäkel, U., Schaich, M., Illmer, T., Pascheberg, U. and Ehninger, G. (2000) "Comparison of

spectral karyotyping and conventional cytogenetics in 39 patients with acute myeloid leukemia and myelodysplastic syndrome", *Leukemia* **14**, 1031–1038.

[24] Kerndrup, G.B. and Kjeldsen, E. (2001) "Acute leukemia cytogenetics: an evaluation of combining G-band karyotyping with multi-color spectral karyotyping", *Cancer Genetics and cytogenetics* **124**, 7–11.

[25] Zhang, F.F., Murata-Collins, J.L., Gaytan, P., Forman, S.J., Kopecky, K.J., Willmann, C.L., Appelbaum, F.R. and Slovak, M.L. (2000) "Twenty-four-color spectral karyotyping reveals chromosome aberrations in cytogenetically normal acute myeloid leukemia", *Genes, Chromosomes, and Cancer* **28**, 318–328.

[26] Hilgenfeld, E., Padilla-Nash, H., McNeil, N., Knutsen, T., Montagna, C., Tchinda, J., Horst, J., Ludwig, W.D., Serve, H., Büchner, T., Berdel, W.E., Schröck, E. and Ried, T. (2001) "Spectral karyotyping and fluorescence *in situ* hybridization detect novel chromosomal aberrations, a recurring involvement of chromosome 21 and amplification of the MYC oncogene in acute myeloid leukaemia M2", *British Journal of Haematology* **113**, 305–317.

[27] Andersen, M.K. and Pedersen-Bjergaard, J. (2001) "Multi-Color FISH in 54 patients with therapy-related MDS/AML", *Blood* **98**, 579a.

[28] Brown, J., Jawad, M., Twigg, S.R., Saracoglu, K., Sauerbrey, A., Thomas, A.E., Eils, R., Harbott, J. and Kearney, L. (2002) "A cryptic t(5;11)(q35;p15.5) in 2 children with acute myeloid leukaemia with apparently normal karyotypes, identified by a multiplex fluorescence *in situ* hybridisation assay", *Blood* **99**, 2526–2531.

[29] Dalley, C.D., Neat, M.J., Foot, N.J., Burridge, M., Byrne, L., Amess, J.A., Rohatiner, A.Z., Lister, A., Young, B.D. and Lillington, D.M. (2002) "Comparative genomic hybridisation and multiplex-fluorescence *in situ* hybridisation: an appraisal in elderly patients with acute myelogeneous leukaemia", *The Hematology Journal* **3**, 290–298.

[30] Ketterling, R.P., Wyatt, W.A., VanWier, S.A., Law, M., Hodnefield, J.M., Hanson, C.A. and Dewald, G.W. (2002) "Primary myelodysplastic syndrome with normal cytogenetics: utility of 'FISH panel testing' and M-FISH", *Leukemia Research* **26**, 235–240.

[31] Klaus, M., Haferlach, T., Schnittger, S., Hiddemann, W. and Schoch, C. (2002) "Acute myeloid leukemia (AML) with normal karyotype: further characterization by fluorescence *in situ* hybridization (FISH) in 489 patients", *Blood* **100**, 310a.

[32] Schoch, C., Chistodoulou, J., Hiddemann, W. and Haferlach, T. (2002) "A distinct pattern of gained and lost chromosomal regions emerges in acute myeloid leukaemia (AML) with complex aberrant karyotype: a study on 41 patients analyzed with 24 color FISH and comparative genomic hybridization(CGH)", *Blood* **100**, 308a.

[33] Vey, N., Groulet, A., Mozziconacci, M.-J., Debono, S., Deraco, S., Devilard, E., Toiron, Y., Tagett, R., Sainty, D., Hermitte, F., Birg, F., Blaise, D., Houlgatte, R., Fert, V., Birnbaum, D. and Bertucci, F. (2002) "Gene expression profiling of acute myeloid leukemias with normal karyotype", *Blood* **100**, 745a.

[34] Lindvall, C., Nordenskjöld, M., Porwit, A., Björkholm, M. and Blennow, E. (2001) "Molecular cytogenetic characterization of acute myeloid leukemia and myelodysplastic syndromes with multiple chromosome rearrangements", *Haematologica* **86**, 1158–1164.

[35] Odero, M.D., Carlson, K.M., Calasanz, M.J. and Rowley, J.D. (2001) "Further characterization of complex chromosomal rearrangements in myeloid malignancies: spectral karyotyping adds precision in defining abnormalities associated with poor prognosis", *Leukemia* **15**, 1133–1136.

[36] Barouk-Simonet, E., Soenen-Cornu, V., Roumier, C., Cosson, A., Lai, J.-L., Fenaux, P. and Preudhomme, C. (2001) "Role of multiplex FISH (M-FISH) in identifying chromosome involvement in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) with complex karyotype. A report on 20 cases", *Blood* **98**, 847a.

[37] Mrózek, K., Heinonen, K., Theil, K.S. and Bloomfield, C.D. (2002) "Spectral karyotyping in patients with acute myeloid leukemia and a complex karyotype shows hidden aberrations, including recurrent overrepresentation of 21q, 11q, 22q", *Genes, Chromosomes, and Cancer* **34**, 137–153.

[38] Schoch, C., Haferlach, T., Bursch, S., Gerstner, D., Schnittger, S., Dugas, M., Kern, W., Löffler, H. and Hiddemann, W. (2002) "Loss of genetic material is more common than gain in acute myeloid leukemia with complex aberrant karyotype: a detailed analysis of 125 cases using conventional chromosome analysis and fluorescence *in situ* hybridization including 24-color FISH", *Genes, Chromosomes, and Cancer* **35**, 20–29.

[39] Van Limbergen, H., Poppe, B., Michaux, L., Herens, C., Brown, J., Noens, L., Berneman, Z., De Bock, R., De Paepe, A. and Speleman, F. (2002) "Identification of cytogenetic subclasses and recurring chromosomal aberrations in AML and MDS with complex karyotypes using M-FISH", *Genes, Chromosomes, and Cancer* **33**, 60–72.

[40] Sait, S.N.J., Qadir, M.U., Conroy, J.M., Matsui, S.-I., Nowak, N.J. and Baer, M.R. (2002) "Double minute chromosomes in acute myeloid leukaemia and myelodysplastic syndrome: Identification of new amplification regions by fluorescence *in situ* hybridisation and spectral karyotyping", *Genes, Chromosomes, and Cancer* **34**, 42–47.

[41] Ning, Y., Liang, J.C., Nagarajan, L., Schröck, E. and Ried, T. (1998) "Characterization of 5q deletions by subtelomeric probes and spectral karyotyping", *Cancer Genetics and Cytogenetics* **103**, 170–172.

[42] Odero, M.D., Carlson, K., Calasanz, M.J., Lahortiga, I., Chinwalla, V. and Rowley, J.D. (2001) "Identification of new translocations involving ETV6 in hematologic malignancies by fluorescence *in situ* hybridisation and spectral karyotyping", *Genes, Chromosomes, and Cancer* **31**, 134–142.

[43] Farag, S., Challis, J., White, J. and Garson, O.M. (1992) "Novel translocation (2;4) with consistent involvement of 2p23 in acute nonlymphocytic leukemia (M2)", *Cancer Genetics Cytogenetics* **58**, 48–51.

[44] Hilgenfeld, E., Padilla-Nash, H., Serve, H., Yonescu, R., Ludwig, W.D., Büchner, T., Berdel, W.E., Ried, T. and Schröck, E. (1999) "Detection of new aberrations in acute myeloid leukemia (AML) by spectral karyotyping (SKY)", *Proceedings of the American Association for Cancer Research* **40**, 541.

[45] Mitelman, F., Johansson, B., Mertens, F., (2002) Mitelman database of chromosome aberrations in Cancer. http://cgap.nci.nih.gov/chromosomes/mitelman

[46] Liyanage, M., Coleman, A., du Manoir, S., Veldman, T., McCormack, S., Dickson, R.B., Barlow, C., Wynshaw-Boris, A., Janz, S., Wienberg, J., Ferguson-Smith, M.A., Schröck, E. and Ried, T. (1996) "Multicolour spectral karyotyping of mouse chromosomes", *Nature Genetics* **14**, 312–315.

[47] Knutsen, T. and Ried, T. (2000) "SKY: a comprehensive diagnostic and research tool. A review of the first 300 published cases", *Journal of the Association of Genetic Technologists* **26**, 3–15.

[48] Jentsch, I., Adler, I.D., Carter, N.P. and Speicher, M.R. (2001) "Karyotyping mouse chromosomes by multiplex-FISH (M-FISH)", *Chromosome Research* **9**, 211–214.

[49] Castilla, L.H., Garrett, L., Adya, N., Orlic, D., Dutra, A., Anderson, S., Owens, J., Eckhaus, M., Bodine, D. and Liu, P.P. (1999) "The fusion gene Cbfb-MYH11 blocks myeloid differentiation and predisposes mice to acute myelomonocytic leukaemia", *Nature Genetics* **23**, 144–146.

[50] Zimonjic, D.B., Pollock, J.L., Westervelt, P., Popescu, N.C. and Ley, T.J. (2000) "Acquired, non-random chromosomal abnormalities associated with the development of acute promyelocytic leukaemia in transgenic mice", *Proceedings of the National Academy of Sciences of the U.S.A.* **97**, 13306–13311.

[51] Changou, A.C., Hilgenfeld, E., McNeil, N., He, L.-Z., Montagna, C., Ried, T. and Pandolfi, P.P. (2001) "A recurring, nonrandom pattern of chromosomal aberrations associated with the development of acute promyelocytic leukemia in PLZF-RARα- and PLZF-RARα/RARα-PLZF transgenic mice", *Blood* **98**, 99a.

[52] Le Beau, M.M., Bitts, S., Davis, E.M. and Kogan, S.C. (2002) "Recurring chromosomal abnormalities in leukaemia in *PML-RARA* transgenic mice parallel human acute promyelocytic leukaemia", *Blood* **99**, 2985–2991.

[53] Sawyer, J.R., Lukacs, J.L., Munshi, N., Desikan, K.R., Singhal, S., Mehta, J., Siegel, D., Shaughnessy, J. and Barlogie, B. (1998) "Identification of new non-random translocations in multiple myeloma with multicolour spectral karyotyping", *Blood* **92**, 4269–4278.

[54] Elghezal, H., Le Guyader, G., Radford-Weiss, I., Perot, C., Van Den Akker, J., Eydoux, P., Vekemans, M. and Romana, S.P. (2001)